REMARKS

This submission is in response to the Official Action dated January 14, 2002. Claims 63-111 have been amended. Claims 1-18, and 63-111 are pending. Claims 1-18 has been withdrawn from further consideration by the Examiner under 37 C.F.R. 1.142(b). Reconsideration of the above identified application, in view of the above amendments and the following remarks, is respectfully requested.

Claims 63-111 have been amended to recite isolated galactose oxidase. This is supported throughout the specification, *e.g.*, at page 21, lines 14-29, and page 25, lines 23-25.

Claims 63-110 have been amended to recite to correlate the amino acid mutations to certain positions in SEQ ID NO:10 or 18 (galactose oxidase amino acid sequences). These amendments are supported throughout the specification, *e.g.*, at page 7, lines 10-11; page 20, line 22 to page 21, line 7; and by FIGS. 17-28.

No new matter has been added by way of this amendment. Each rejection is discussed in turn below.

Priority

The Examiner has contended that the specification does not contain a specific reference to the priority application, U.S. 09/571,553, filed May 16, 2000.

The Examiner's attention is respectfully directed to the preliminary amendment contained in the transmittal letter accompanying the present patent

application filed on November 27, 2000, amending the specification to refer to the priority application.

This objection is therefore moot and should be withdrawn.

Rejection Under 35 U.S.C. 101

The Examiner has rejected claims 63-111 for being directed to non-statutory subject matter.

As amended, claims 63-111 call for isolated galactose oxidase. This rejection has therefore been overcome and should be withdrawn.

Rejections Under 35 U.S.C. 112, 1st Paragraph

Written Description

The Examiner has rejected claims 63-111 as allegedly not meeting the written description requirement. The Examiner alleges that the specification discloses only one representative species, mutant galactose oxidase of *D. denroides* or *Fusarium*, and that this is not enough to describe the whole genus.

Applicants respectfully disagree.

The Federal Circuit recently clarified the written description requirement in *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, No. 01-1230, 2002 U.S. App. LEXIS 5642 (Fed. Cir. 2002). "An adequate written description of genetic material 'requires a precise definition, such as by structure, formula, chemical name, or physical

properties.'" 2002 U.S. App. LEXIS 5642, at *10 - *11 (citation omitted). When a functional characteristic is used to substantiate a precise definition, "the claimed function [must be] known to correlate to a specific structure or other identifying characteristic that is disclosed or is otherwise well known." *Id.* at *14. Here, the specification discloses unifying structural and functional features which describe the invention as claimed.

As amended, claims 63-110 recite amino acid mutations in residues corresponding to specific residues in either SEQ ID NOS:10 or 18. SEQ ID NO:10 is a galactose oxidase sequence differing from wild-type galactose oxidase by an N to D amino acid mutation at position 537 (FIG. 17), and SEQ ID NO:18 is a galactose oxidase sequence differing from wild-type galactose oxidase by an N to D amino acid mutation at position 413 (FIG. 25). Claim 111 recites specific amino acid sequences selected from SEQ ID NOS:10-21.

Applicants respectfully submit that these amendments now provide precise written description of both structural and functional bases premised upon the identification of specific structures.

The enzymes of the claimed invention all have the same unifying function; galactose oxidase activity, *i.e.*, they are all enzymes which promote oxidation of sugars and alcohols, accepting D-galactose as a substrate. This function is described in detail in the specification (*e.g.*, at page 4, line 5 to page 6, line 16; and page 26, lines 7-25), and methods for assaying the same are described throughout the

specification. In particular the Examples describe methods for high-throughput screening of galactose oxidases (see, *e.g.*, Example 1, page 27, line 8 to page 34, line 20) to identify galactose oxidase activity.

The galactose oxidases genus is a particularly well-defined group with known structural features (page 4, lines 5-27). The amended claims correlate the structural features of the variant cytochrome P450 enzymes of the invention with those of the galactose oxidases described in the specification, providing a reference point against which the enzyme genus of the invention is clearly described and distinctly defined. Accordingly, for amended claims 63-110, the amino acid sequence for a variant galactose oxidase enzyme, prepared as described in the Examples, can be aligned with SEQ ID NO:10 or 18 as a reference sequence, using a suitable sequence alignment algorithm, such as, *e.g.*, MEGALIGN or BLAST (see specification, page 20, line 22 to page 21, line 7) to identify which residue corresponds to the positions of the specific mutations set forth in the claims.

As an illustration of such sequence alignments and the high level of knowledge in the art at the time of filing the instant application, the Examiner's attention is respectfully directed to Exhibit A; a journal article by Whittaker et al. (J Biol Chem 274:36226-36232; "Whittaker"), published December 17, 1999. The publication describes alignment and comparison of amino acid sequences for a galactose oxidase enzyme and a glyoxal oxidase enzyme (Whittaker, page 36229, Figure 2), and identifies aligned residues at five positions in the sequences. Thus,

even though Whittaker relates to a glyoxal oxidase sequence aligned with a galactose oxidase, the reference shows that such sequence alignments and how to find amino acid or nucleotide positions corresponding to certain residues in a reference galactose oxidase sequence was, and has long been, well known in the art at the time of filing the instant application.

In summary, the claimed invention is defined both by structural and functional features, coupled in a way that uniquely identifies and describes the subject matter of the invention. Therefore, Applicants respectfully requests reconsideration and withdrawal of this rejection.

Enablement

The Examiner has rejected claims 63-111 as allegedly not enabled by the specification. The Examiner contends that the specification enables a mutant galactose oxidase from *D. dendroides*, but not mutant/variant galactose oxidase enzymes from other organisms. According to the Examiner, it is not routine to screen large numbers of amino acid sequences where the expectation of obtaining similar sequences is unpredictable, and knowledge of which sequences can be altered is outside of the realm of routine experimentation.

As amended, the claims call for galactose oxidase enzymes which have mutations at positions corresponding to the recited positions of SEQ ID NOS:10 or 18 (claims 63-110); and which have specific amino acid sequences (claim 111). These

embodiments of the invention are all enabled by the specification. A person of ordinary skill can make and use the claimed invention according to the guidance in the specification, without undue experimentation. As explained in *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404, (Fed. Cir. 1988) (citing In Re Angstadt, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19, (CCPA 1976)):

> To be enabling, the specification of the patent must teach those skilled in the art how to make and use the full scope of the claims of invention without 'undue experimentation.' Genentech Inc. v. Novo Nordisk, A/S, 42 USPQ 2d 1001, 1004 (Fed. Cir. 1997) (quoting In re Wright, 27 USPQ 2d 1510, 1513 (Fed. Cir. 1993)). The court has held that a patent specification complies with a statute even if a 'reasonable' amount of routine experimentation is required but such experimentation must not be 'undue.' [A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977). 'The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.'

The claimed galactose oxidases have defined structures according to disclosed sequence information, and are all characterized by having galactose oxidase activity. The specification, in particular the Examples, provides ample guidance on methods for creating, high-throughput screening for, and characterizing of, galactose oxidases. Thus, the galactose oxidase activity, modification tolerance and whether a galactose oxidase enzyme would be successful can be assessed by the provided high-throughput screening methods. Parent molecules are well described, as are mutation and expression techniques. Assay methods for galactose oxidase activity and other

properties are described in detail, as are high throughput screening methods to efficiently identify active variants. Although time-consuming experimentation may be involved, such experimentation would be reasonable and not undue, since the specification provides sufficient guidance and direction, particularly in the Examples.

In addition, the galactose oxidase amino acid sequences, prepared as described in the Examples, can be aligned with the recited reference sequences (SEQ ID NOS:10 or 18) using a suitable sequence alignment algorithm (see specification, page 20, line 22 to page 21, line 7) to identify which residue corresponds to the positions of the specific mutations set forth in claims 63-110. Claim 111 recites specific amino acid sequences. While sequence alignments might be time consuming if a large number of sequences are compared, such experimentation is routine and would not be "undue" or lacking enablement under § 112.

The *In Re Wands* factors referred to by the Examiner are thus met by the instant application, as follows:

(1) *The quantity of experimentation necessary*. The quantity of experimentation, while perhaps extended, is not excessive and is lessened by the high-throughput screening methods in the Examples coupled with automated sequencing and simple sequence alignment methods currently available. Contrary to the Examiner's contentions, it is routine to screen large numbers of sequences for common properties, such as enzyme activity, particularly if the sequences are derived from a common parent or have a common evolutionary origin.

(2) *The amount of direction or guidance presented*. The specification provides ample guidance which practitioners can readily follow to practice the invention, including the high-throughput screening methods and sequence alignment methods, as well as to galactose oxidase activity and specificity.

(3) *The presence or absence of working examples*. Working examples for preparing, screening for, and identifying the galactose oxidase enzymes of the invention are described in Examples 1-3.

(4) *The nature of the invention*. Practitioners recognize and accept that there is some unpredictability in the field of genetic engineering, and consider screening techniques like those disclosed to be routine and reasonable discriminatory tools. Here, there is specific guidance for using these tools to practice the claimed invention.

(5) and (6) *The state of the prior art and the level of skill in the art*. These are represented by the Whittaker publication (Exhibit A), showing for example a sequence alignment with a reference galactose oxidase and identification of corresponding residues.

(7) *The predictability and unpredictability of the art*. While there may be a certain unpredictability in the creation of protein variants using directed evolution, the directed evolution and high-throughput methods described herein enable the creation and screening of a large number of mutants with a reasonable expectation of success.

(8) *The breadth of the claims*. As amended herewith, the claims describe and distinctly claim both structural and functional features of the galactose oxidase enzymes of the invention, and are not unduly broad.

Accordingly, the invention as set forth by the claims is enabled. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

Rejection Under 35 U.S.C. 112, 2nd Paragraph

The Examiner has rejected claims 63-111 for alleged indefiniteness. Specifically, the Examiner contends that absent a reference sequence, it is not clear which residues have, in fact, been mutated.

With this amendment, claims 63-110 refer to one or more reference sequences selected from SEQ ID NO:10 and 18. Thus, these claims are directed to variant enzymes which have mutations at positions corresponding to the recited positions of SEQ ID NO:10 or 18, which differ from wild-type galactose oxidase by one mutation. Claim 110 calls for specific galactose oxidase sequences.

As discussed above, the amino acid sequence for a variant galactose oxidase enzyme, prepared as described in the Examples, can be aligned with SEQ ID NO:10 or 18 as a reference sequence, using a suitable sequence alignment algorithm, such as, *e.g.*, MEGALIGN (see specification, page 20, line 22 to page 21, line 7) to identify which residue corresponds to the positions of the specific mutations set forth in the claims.

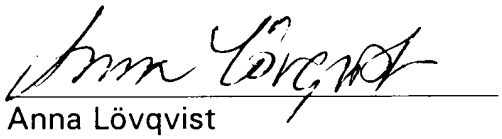
This rejection has therefore been overcome and should be withdrawn.

* * *

Therefore, in view of the above amendments and remarks, it is respectfully requested that the application be reconsidered and that all pending claims be allowed and the case passed to issue.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



Anna Lövqvist
Limited Recognition Under
37 C.F.R. 10.9(b) (See attached)
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PATENT TRADEMARK OFFICE

Docket No: 4058/1G811US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Frances H. ARNOLD, et al.

Serial No.: 09/722,602 Art Unit: 1652

Confirmation No.: 5781

Filed: November 27, 2000 Examiner: Y. PAK

For: DIRECTED EVOLUTION OF OXIDASE ENZYMES

MARK-UP ACCOMPANYING AMENDMENT
PURSUANT TO 37 C.F.R. §1.121

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

April 15, 2002

Sir:

IN THE CLAIMS:

Please amend the claims pursuant to 37 C.F.R. 1.121 as follows:

63. (Amended) [A] An isolated galactose oxidase which has a mutation in at least one amino acid corresponding to an amino acid selected from the group

consisting of A3, S10, M70, P136, G195, T218, L312, [N413,] V494, C515, N535, N537, [S550,] and S610 of SEQ ID NO:18 and N413 and S550 of SEQ ID NO:10.

64. (Amended) [A] An isolated galactose oxidase which has at least one of the amino acid mutations corresponding to S10P, M70V, G195E, [N413D,] V494A, C515S, N535D, and N537D of SEQ ID NO:18 and N413D of SEQ ID NO:10.

65. (Amended) The isolated galactose oxidase of claim 64, which has the amino acid mutation corresponding to N537D of SEQ ID NO:18.

66. (Amended) The isolated galactose oxidase of claim 64, which has the amino acid mutation corresponding to V494A of SEQ ID NO;18.

67. (Amended) The isolated galactose oxidase of claim 66, further comprising the amino acid mutation corresponding to C515S of SEQ ID NO:18.

68. (Amended) The isolated galactose oxidase claim 66, further comprising the amino acid mutation corresponding to S10P of SEQ ID NO:18.

69. (Amended) The isolated galactose oxidase of claim 66, further comprising a silent mutation at a position corresponding to P136 of SEQ ID NO:18.

70. (Amended) The isolated galactose oxidase of claim 68, further comprising a silent mutation at a position corresponding to P136 of SEQ ID NO:18.

71. (Amended) The isolated galactose oxidase of claim 66, further comprising the amino acid mutation corresponding to G195E of SEQ ID NO:18.

72. (Amended) The isolated galactose oxidase of claim 71, further comprising a silent mutation in at least one of positions corresponding to A3 and P136 of SEQ ID NO:18.

73. (Amended) The isolated galactose oxidase of claim 66, further comprising the amino acid mutation corresponding to N535D of SEQ ID NO:18.

74. (Amended) The isolated galactose oxidase of claim 73, further comprising a silent mutation in at least one of positions corresponding to P136, L312, and T218 of SEQ ID NO:18.

75. (Amended) The isolated galactose oxidase of claim 66, further comprising the amino acid mutation corresponding to M70V of SEQ ID NO:18.

76. (Amended) The isolated galactose oxidase of claim 75, further comprising a silent mutation at a position corresponding to P136 of SEQ ID NO:18.

77. (Amended) The isolated galactose oxidase of claim 64, which has the amino acid mutations corresponding to S10P, M70V, G195E, V494A and N535D of SEQ ID NO:18.

78. (Amended) The isolated galactose oxidase of claim 77, further comprising a silent mutation at a position corresponding to P136 of SEQ ID NO:18.

79. (Amended) The isolated galactose oxidase of claim 64, which has the amino acid mutation corresponding to N413D of SEQ ID NO:10.

80. (Twice amended) The isolated galactose oxidase of claim 79, further comprising a silent mutation at a position corresponding to S550 of SEQ ID NO:10.

81. (Amended) The isolated galactose oxidase of claim 66, further comprising the amino acid mutation corresponding to N413D SEQ ID NO:10.

82. (Amended) The isolated galactose oxidase of claim 81, further comprising a silent mutation in at least one of a position corresponding to S550 and S610 of SEQ ID NO:10.

83. (Amended) [A] An isolated galactose oxidase which has a mutation in at least one amino acid corresponding to a position selected from the group consisting of A3, S10, M70, P136, T218, L312, [N413,] C515, N535, N537, S550, and S610 of SEQ ID NO:18 and N413 of SEQ ID NO:10.

84. (Amended) The isolated galactose oxidase of claim 83, further comprising at least one amino acid mutation corresponding to a mutation selected from the group consisting of G195 and V494 of SEQ ID NO:18.

85. (Amended) The isolated galactose oxidase of claim 83, wherein the mutation is selected from a mutation corresponding to one of the group consisting of S10P, M70V, [N413D,] C515S, N535D, and N537D of SEQ ID NO:18 and N413D of SEQ ID NO:10.

86. (Amended) The isolated galactose oxidase of claim 85, further comprising at least one amino acid mutation corresponding to a mutation selected from the group consisting of G195E and V494A of SEQ ID NO:18.

87. (Amended) [A] An isolated galactose oxidase which has a mutation in an amino acid corresponding to N537 of SEQ ID NO:18.

88. (Amended) The isolated galactose oxidase of claim 87, wherein the mutation is N537D.

89. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494 and C515 of SEQ ID NO:18.

90. (Amended) The isolated galactose oxidase of claim 89, wherein the mutations are V494A and C515S.

91. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494 and P136 of SEQ ID NO:18.

92. (Amended) The isolated galactose oxidase of claim 91, wherein the V494 mutation is V494A.

93. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494, P136, and S10 of SEQ ID NO:18.

94. (Amended) The isolated galactose oxidase of claim 93, wherein the V494 mutation is V494A, and the S10 mutation is S10P.

95. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494, P136, G195, and A3 of SEQ ID NO:18.

96. (Amended) The isolated galactose oxidase of claim 95, wherein the V494 mutation is V494A, and the G195 mutation is G195E.

97. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494, P136, L312, N535, and T218 of SEQ ID NO:18.

98. (Amended) The isolated galactose oxidase of claim 97, wherein the V494 mutation is V494A, and the N535 mutation is N535D.

99. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494, P136, and M70 of SEQ ID NO:18.

100. (Amended) The isolated galactose oxidase of claim 99, wherein the V494 mutation is V494A, and the M70 mutation is M70V.

101. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to V494, S10, P136, M70, G195, and N535 of SEQ ID NO:18.

102. (Amended) The isolated galactose oxidase of claim 101, wherein the V494 mutation is V494A, the S10 mutation is S10P, the M70 mutation is M70V, the G195 mutation is G195E, and the N535 mutation is N535D.

103. (Amended) [A] An isolated galactose oxidase which has a mutation in an amino acid corresponding to N413 of SEQ ID NO:10.

104. (Amended) The isolated galactose oxidase of claim 103, wherein the mutation is N413D.

105. (Amended) [A] An isolated galactose oxidase which has a mutation in amino acids corresponding to N413 and S550 of SEQ ID NO:10.

106. (Amended) The isolated galactose oxidase of claim 105, wherein the N413 mutation is N413D.

107. (Amended) [A] An isolated galactose oxidase which has a mutation in amino acids corresponding to N413 of SEQ ID NO:10, and S550[,] and V494 of SEQ ID NO:18.

108. (Amended) The isolated galactose oxidase of claim 107, wherein the N413 mutation is N413D, and the V494 mutation is V494A.

109. (Amended) [A] An isolated galactose oxidase which has mutations in amino acids corresponding to N413 of SEQ ID NO:10, and S550, V494, and S610 of SEQ ID NO:18.

110. (Amended) The isolated galactose oxidase of claim 109, wherein the N413 mutation is N413D, and the V494 mutation is V494A.

111. (Amended) [A] An isolated galactose oxidase having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10-21.